John von Neumann Institute for Computing

Effects of Confinement and Crowding in Protein Folding

M. Wojciechowski, M. Cieplak

published in

From Computational Biophysics to Systems Biology (CBSB08),
Proceedings of the NIC Workshop 2008,
Ulrich H. E. Hansmann, Jan H. Meinke, Sandipan Mohanty,
Walter Nadler, Olav Zimmermann (Editors),
John von Neumann Institute for Computing, Jülich,
NIC Series, Vol. **40**, ISBN 978-3-9810843-6-8, pp. 145-148, 2008.

http://www.fz-juelich.de/nic-series/volume40

Effects of Confinement and Crowding in Protein Folding

Michal Wojciechowski and Marek Cieplak

Institute of Physics, Polish Academy of Sciences,
Al. Lotników 32/46, 02-668 Warsaw, Poland
E-mail: mwoj@ifpan.edu.pl

We consider folding and unfolding of a protein contained within a sphere of radius R. We use a coarse-grained geometry based model. We find that the folding time is essentially independent of R but the possible unfolded structures do depend on R. Several proteins placed within the sphere fold mostly independent of one another unless one introduces attractive interactions between them. Attractive interactions between molecules have strong influence on the folding times.

1 Introduction

Many experimental and theoretical studies of proteins involve very low protein concentrations. In cells, however, the proteins are confined to compartments and come with high concentrations[1]. It is thus interesting to assess the role of confinement and crowding theoretically by first considering simple models.

Previous theoretical studies of confinement[2–6] have been focused on thermodynamics. The confinment has been found to lead to a greater thermodynamic stability, broader and taller specific heat and more compact unfolded conformations[4,3].

Recently, we have studied effects of molecular crowding and caging on protein folding within a simple molecular dynamics model[7]. Here, we provide a brief account of these studies.

2 Model

We used the coarse-grained Go-like model, where amino acids are represented by beads. The beads interact by a potential that maintains chain topology and enforces the local backbone stiffenss through the chirality terms[8]. The native contacts are described by

$$V_{ij}^{contact} = 4\epsilon \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] \tag{1}$$

where r_{ij} describes the distance between the bead centers. By taking $\sigma_{ij} = r_{ij}^{native}/\sqrt[6]{2}$ the potential acquires a minimum at a distance r_{ij}^{native} that is found in the native structure of the protein. The repulsive potential that keeps protein inside the sphere may be written as

$$V_{wall}(s_i) = \begin{cases} 4\epsilon \left[\left(\frac{\sigma}{s_i} \right)^{12} - \left(\frac{\sigma}{s_i} \right)^{6} \right] + \epsilon \ , & s_i < 4 \qquad s_i = R - r_i \\ 0 \qquad\qquad\qquad\qquad\qquad\quad , & s_i \geq 4 \qquad \sigma = 4/\sqrt[6]{2} \end{cases} \tag{2}$$

where r_i is a distance of the ith center to the origin of the sphere, R is the radius of the sphere.

The attractive interaction between separate molecules is introduced in simplified way. When dealing with several identical proteins, it is natural to assume that if a pair of amino acids forms the native contact in one molecule, the same pair of amino acids, but belonging to different molecules, will also interact. The strength, ϵ_I, of such an interaction is an input to the modelling. We take

$$V_{ij'}^{inter} = 4\epsilon_I \left[\left(\frac{\sigma_{ij}}{r_{ij'}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij'}} \right)^{6} \right], \qquad 0 \geq \epsilon_I \geq \epsilon \tag{3}$$

and ϵ_I is considered to be weaker than ϵ from Eq.1. We have determined the folding times, t_{fold}, defined as the median over the set of 301 trajectories. The folding time of a molecule is defined as the first time at which all of its native contacts are established simultaneously. For several molecules, test of the presence of the native contacts are performed for each molecule separately. The simulations were performed for crambin – the small, one domain, α-β protein with the PDB code 1CRN.

3 Results

We computed t_{fold} of crambin in spheres with various radii ranging from $R = 1000$Å down to 18Å (corresponding to the smalest sphere that may fit the crambin in the native state). The starting structures for folding were obtained through thermal unfolding at a high temperature in sphere of radius R_0. To evaluate the influence of the starting structures on t_{fold} we also study folding in an unbounded space. Results of the simulations are presented on Fig.1A.

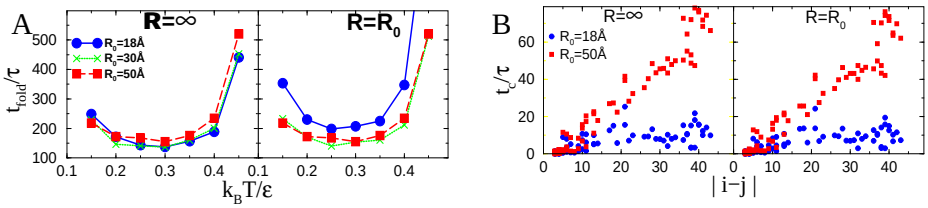

Figure 1. A: Folding time of crambin computed with different starting structure sets (corresponding to various indicated choices of R_0). The left panel shows folding in an infinite space and the right panel in a sphere of a finite radius radius $R = R_0$. B: Folding scenarios of crambin computed with different starting structure set (R_0 describes the radius of sphere in the unfolding simulation that produce the starting structures for folding). Left panel shows folding in infinite space, right panel folding in sphere of radius $R = R_0$ (the same radius as for the unfolding procedure).

There is no influence of the starting structures on t_{fold}, Fig.1A (left panel), and there is only a weak influence the sphere size on t_{fold} as shown in Fig.1A (right panel). Thus the confinement does not change the folding times of proteins unless one considers very tight confinement conditions under which the protein barely moves. We also find that even

though the sequencing of folding events depends on the choice of the starting R_0, the confinement itself does not affect it, see Fig1B.

We now consider the crowding phenomena and place several protein in one 36Å-sphere. We calculate t_{fold} for one, two, eight and twelve molecules. The simplest case is when the only inter-molecular interactions are only due to the excluded volume. The corresponding results are presented in Fig.2A. It is seen that the number of molecules that are present does not change an individual folding time.

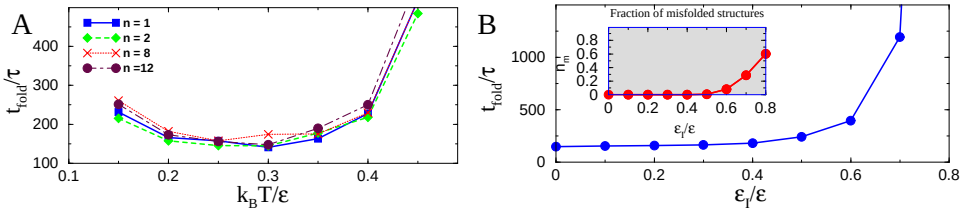

Figure 2. A: Folding times for different number of proteins within the sphere. B: Folding times for four crambins on different interaction strength. The inset shows the fraction of misfolded structures.

The situation changes when attractive inter-molecular interactions are added. Fig.2B shows t_{fold} for R=36 Å. For $\epsilon_I < 0.5\epsilon$, t_{fold} is nearly the same as for ϵ_I=0. However, for larger values of ϵ_I, t_{fold} raises significantly. Eventually, all structures get misfolded, because of aggregation.

Acknowledgments

This work has been supported by the grant N N202 0852 33 from the Ministry of Science and Higher Education in Poland.

References

1. R. J. Ellis and A. P. Minton, *Protein aggregation in crowded environments*, Biol Chem. **387**, 485–97, 2006.
2. A. Baukmketner, A. I. Jewett, and J. E. Shea, *Effect of confinement in chaperonin assisted protein folding: rate enhancement by decreasing the roughness of the folding energy landscape.*, J. Mol. Biol. **332**, 701–13, 2003.
3. N. Rathore, T. A. Knotts IV, and J. J. de Pablo, *Confinement effects on the thermodynamics of protein folding: Monte Carlo simulations*, Biophys J. **90**, 1767–73, 2006.
4. F. Takagi, N. Koga, and S. Takada, *How protein thermodynamics and folding mechanisms are altered by the chaperonin cage: molecular simulations*, Proc. Natl. Acad. Sci. USA **100**, 11367–72, 2003.
5. A. I. Jewett, A. Baumketner, and J.-E. Shea, *Accelerated folding in the weak hydrophobic environment of a chaperonin cavity: creation of an alternate fast folding pathway*, Proc. Natl. Acad. Sci. USA **101**, 13192–7, 2004.

6. M. S. Cheung, D. Klimov, and D. Thirumalai, *Molecular crowding enhances native state stability and refolding rates of globular proteins*, Proc. Natl. Acad. Sci. USA **102**, 4753–8, 2005.
7. M. Wojciechowski and M. Cieplak, *Effects of confinement and crowding on folding of model proteins*, Biosystems (in press), 2008.
8. J. I. Kwiecinska and M. Cieplak, *Chirality and protein folding*, J. Phys. Cond. Mat. **17**, S1565–80, 2005.